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16. ABSTRACT

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Chrysotile Asbestos in Kidney Cortex of Chronically Gavaged Rats

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Abstract. Using the transmission electron microscope, asbestos fibers have been assessed in kidney cortex of four groups of rats previously exposed to intermediate range feeding grade chrysotile asbestos. Newborn rats, from mothers gavaged with asbestos during pregnancy, were gavaged twice a week at the dose level of 50 mg/kg beginning at age day 7 until their natural death or sacrifice. Four groups of rats consisted of ages ranging from 0–200, 200–400, 400–600 and 600–800 days. Of the 20 rats comprising the four test groups, 17 were positive, average fibers recovery being 5.34×10^3/mg dry weight. Average fiber level in control tissues was 0.23×10^3/mg dry weight. Fiber recovery in tissues from control animals was shown to be significantly lower ($P < 0.005$) than that from test tissues. Test groups showed highly significant differences ($P < 0.005$) from each other in the fiber levels. Dose-response relationship was not significant ($0.05 < P < 0.1$). The length distribution and the alterations in morphology of the recovered fibers are described. This study is consistent with the passage of chrysotile asbestos across the gastrointestinal wall.

Chrysotile asbestos accounts for about 95% of asbestos used commercially in the U.S. (Speil and Leineweber 1969). It is ubiquitously present in the environment and is a proven carcinogen to occupationally exposed humans. There has been concern about the health effect, particularly carcinogenic, of asbestos ingested by the general population due to the presence of the mineral in foods and drugs (Maggiore 1974; Fed. Reg. 1978), beverages (Cunningham and Pontefract 1971) and drinking water (Cunningham and Pontefract 1971; Kay 1974; Kanarek 1978; Millette *et al.* 1980). Detectable levels of asbestos in 45% of water samples from 365 cities (Millette *et al.* 1980) and fiber counts up to 136 million/L in Bay Area counties of California and 143 million/L in Everett, Washington (Kanarek 1978) were reported.

Some epidemiological data (Keal 1960; Selikoff *et al.* 1964; Enterline and Kendrick 1967; Selikoff 1974) suggest a relationship between ingested asbestos and cancer incidence in humans. Long-term animal feeding studies have been conducted recently at several research centers to investigate whether or not malignant tumors are developed in animals chronically fed with asbestos; some results are now available (Donham *et al.* 1980; Sebastien *et al.* 1980; McConnell *et al.* 1981; Hilding *et al.* 1981; Epstein and Iqbal 1982). Human studies (Langer *et al.* 1971; Selikoff *et al.* 1979; Cook and Olson 1979; Carter and Taylor 1980) and short-term animal feeding studies (Cunningham *et al.* 1977; Patel-Mandlik *et al.* 1979; 1980; Sebastien *et al.* 1980) have shown that ingested fibers penetrate, migrate between, and are recoverable from gastrointestinal tract, lymph, distant tissues, and urine.

The objective of the present study was to analyze tissues of rats employed in a long-term feeding study (National Cancer Institute grant 21684), to gather information on the retention of penetrated and migrated fibers. Kidney cortex was selected, since it was reported to retain the highest concentration of ingested asbestos among the ten analyzed organs (Patel-Mandlik 1980).

The mechanism of the carcinogenicity of asbestos has not been fully explored and the information on the concentration and physicochemical states of fibers in the sites of primary importance or suspected cancer target organs is still quite limited. Selikoff *et al.* (1979) reported that the post-mortem operations

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of insulation workers revealed numerous asbestos fibers in their livers, but cancer excesses occurred in the kidney rather than the liver. He reported that the occurrence of kidney cancer was two to three times higher among the workers. In our study, the relationship was tested between the time of exposure to a high concentration of ingested asbestos (cumulative gavages) and the concentration of chrysotile fibers recovered in the kidney cortex. In addition, omentum and lung from one test rat were analyzed to test the presence of fibers in those organs.

Materials and Methods:

Source of Tissues: Tissues were collected from Sprague Dawley rats employed in a National Cancer Institute supported study on carcinogenicity of long-term feeding of asbestos to rats and mice. Rats were selected of the group exposed to high dose (50 mg/kg) of Environmental Health Sciences Sample No. 109C feeding grade intermediate range chrysotile asbestos. The rats were the progeny of 13 mothers who were orally administered chrysotile asbestos suspension during their pregnancy. The level of asbestos was determined by electron microscopy in kidney cortex of 20 male and female rats randomly selected from 120 first generation progeny. Control mothers and F_1 progeny rats were orally administered with an equal aliquot of water without asbestos following the same schedule of doses as the test animals. Four control rats, of 127 F_1 progeny rats were randomly chosen to represent the level of asbestos in control tissues. Four age groups were formed, for convenience. The four age groups consisted of rats in the age ranges of 0–200 days, 200–400 days, 400–600 days, and 600–800 days. All rats were given food and water *ad libitum* and were chronically gavaged twice per week beginning at age day 7 until their natural death or sacrifice. During autopsy, the organs were removed, washed, and preserved in formaldehyde or buffered formalin.

Tissue Preparation and Analysis: Specimens of kidneys were thoroughly washed with filtered (0.1 μm) distilled water. The cortex and medulla of the kidney were separated and pieces of cortex from different areas were used for chrysotile asbestos analysis by transmission electron microscopy (TEM). The samples were prepared according to the method used previously (Patel-Mandlik and Millette 1980). The fibers were counted at 80 kv and 23,000× magnification. A criterion of 50 fibers or 50 grid holes, whichever came first, was used per sample or two grids. Fiber dimensions were measured at 26,000× magnification on the inscribed scale of a Philips 300 electron microscope. The size determination of bundles was based on the length of the longest fiber and combined width of a bundle and, that of clusters on the sizes of fibrils and bundles within a cluster. Randomly selected grids (about 10% of the total number of grids) were sent to the Health Effects Laboratory of U.S. Environmental Protection Agency, Cincinnati, Ohio, for fiber count and size analysis.

Chrysotile was identified by the characteristic morphology, aspect ratio of 3:1 and selected area electron diffraction pattern. However, not all of the particles confirmed as chrysotile by morphology could meet the fiber criterion of an aspect ratio of 3:1 because of internal channel swelling. Also, the degradative alterations in the fiber made it difficult to obtain a chrysotile diffraction pattern on a number of fibers. For the purpose of descriptive state of fiber morphology, four categories were formed arbitrarily: Class A, Class B, Class C, and Class D fibers. The following definitions were flexibly used to place a fiber in its appropriate class, since a personal judgement was required, especially in case of degraded fibers:

Class A — Chrysotile fibers showing characteristic appearance and a distinct central canal (Figure 1).

Class B — Fibers with tubular morphology. Central canal not visible through the entire length of the fiber (Figure 2).

Class C — Fibers with degraded morphology yet with partial or complete central canal (Figure 3a) as well as fibers showing more or less characteristic appearance of chrysotile and fairly clear central canal in some if not all fibers in a bundle or cluster (Figure 3b).

Class D — Highly degraded fibers: though not with definite chrysotile appearance or central canal, yet showing identity well enough to place them as chrysotile asbestos fibers (Figure 4).

In addition to kidney cortex from 20 test rats, samples of lung and omentum from one test rat, age 842 days, were analyzed.

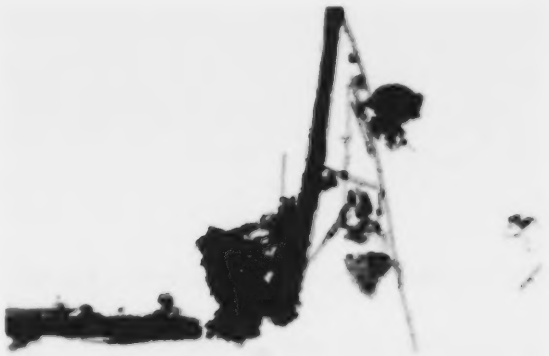

Fig. 1. Class A chrysotile asbestos fibers recovered in kidney cortex of test rat. The central canal and tubular appearance characteristic of chrysotile are clearly seen. ×29,000

Fig. 2. Micrograph showing Class B fibers in a sample from rat kidney cortex. Fibers have tubular appearance. Central canal is visible in part of the fiber length only. ×29,000

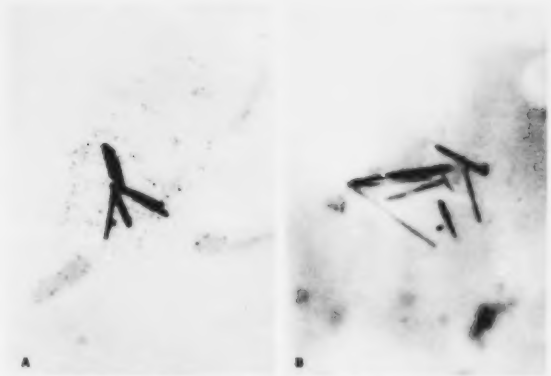

Fig. 3. (A) Degraded fibers though swollen have visible central canal. The source was a gavaged rat's kidney cortex. (B) A cluster showing characteristic appearance of chrysotile, but central canal is clearly visible in only one fiber. Photograph of a preparation from rat kidney cortex. (A) and (B) represent Class C fibers. ×29,000

Fig. 4. Class D fiber, recovered from rat kidney cortex, reveals further degradation of characteristic morphology of chrysotile asbestos. The central canal is almost absent. ×58,000

Using the same preparative and counting techniques, blank preparations and tissue preservative were tested for the background level of fibers.

Asbestos fiber concentrations were calculated by a previously published equation (Patel-Mandlik and Millette 1980), the only difference being that in this study, C (the number of grid holes examined), was not a constant number (Table 1). Hence, the equation was slightly changed as follows:

$$\frac{\text{Fibers per grid hole} \times 4375}{L} \text{ where } \frac{A_1 \times V_1}{A_2} = 4375.$$

Sample preparation steps were designed to achieve the tissue load (L) on the TEM grid surface within very narrow limits (see $M \times V_2$ in Table 1). The detection limit of this technique was 88 fibers/mg dry weight if one fiber were found in 50 grid holes and if L equalled 1.

Results

There was little trouble in transmission electron microscope preparation of tissues. The residue on grids was minimal, leaving the fibers unobscured for identification, counting, and sizing. Fiber distribution on the surface of the grid was tested statistically and found to be Poissonian (Cochran 1954).

Pooled numerical values of fiber recovery are shown in Table 2. A total of 788 fibers were encountered in the test samples. Fibers of designated Classes A, B, and C (Figures 1–3) were recorded and are reported in Tables 2 and 3. Fibers of Class D (Figure 4) were recorded in analysis of five randomly selected grids only. Table 3 shows concentrations calculated for Class A + B and Class A + B + C fiber counts. The numbers of fibers/mg dry weight were found to be highest in samples from rats in age range 600 to 800 days. Tissues from rats in age range 0 to 200 days showed the lowest concentration. In terms of organ load, one can convert the above values of fibers/mg dry weight of kidney cortex into fibers per kidney. The average wet weights of one kidney each of 100, 300, 500 and 700 days old rats were 1.23, 1.72, 1.87 and 2.04 g, respectively. These values represent the midpoint of the age-ranges of the four groups of rats in the study.

The tissues from control animals indicated below detection (B.D.) levels of Class A + B fibers. Class C fibers were found in only two control tissues to an average value of 200 fibers/mg dry weight. Such insignificant recovery of only C fibers in the controls provided confidence in the true presence of fibers in the test tissues. Fibers were at B.D. level in blank grids and grids prepared from tissue preservative. Examination of blank grids (prepared by filtering distilled water) was a test for contamination from glassware, water, nuclepore, laboratory room air, formvar-carbon coated TEM grids, reagents, and syringe. Optical microscopy of airborne asbestos in rooms housing the control rats and test rats showed a highly significant difference ($P < 0.01$) in fiber levels. Comparison of air asbestos levels measured by optical microscopy, in the cage area and dosing area of the test animals' room showed a significant difference ($P < 0.05$).

For statistical comparisons square root transformations of data from tissue analyses were used, because the mean and the variance were proportional. Fiber recovery in test and control groups were compared (Sokal and Rohlf 1973) in each of the four time intervals; significant difference at $P < 0.005$ was found. The individual time interval transformed data were combined to test an overall difference, which was at $P < 0.005$.

Dose-response effect was tested by comparing the fiber recovery in test samples from the four age groups. Group I animals represented the lowest dosage of gavaged asbestos and group IV animals

Table 1. Description of test and control rats and of grids analyzed by transmission electron microscope (TEM)

Rat group by age	Rat[a] ID	Age (Days)	Sex	Tissue (mg) per grid surface ($M \times V_s$)[b,c]	Grid holes examined[d]
Group I		65	M	1.004	37
(0–200 days)	T	152	M	1.005	50
	E	192	M	1.000	50
	S	173	F	1.042	50
	T	121	M	1.016	50
	Control	119	M	1.000	50
Group II		300	M	1.000	50
(200–400	T	304	M	0.999	50
days)	E	204	F	1.002	26
	S	227	M	1.005	50
	T	264	M	1.018	50
	Control	334	M	0.998	50
Group III		449	F	1.735	50
(400–600	T	501	F	1.004	37
days)	E	567	M	1.000	40
	S	537	M	1.036	50
	T	596	F	0.976	12
	Control	442	F	1.001	50
Group IV		723	M	1.035	50
(600–800		694	F	1.001	50
days)		710	F	1.128	50
		617	F	1.023	41
		637	M	0.998	38
	Control	800	M	1.040	50

[a] Dosage = 50 mg/kg twice a week from birth till death
[b] M = mg dry wt of tissue in suspension
[c] V_s = μl volume of suspension deposited per grid surface
[d] 50 grid holes or 50 fibers whichever comes first was the criterion in TEM scanning of 2 grids per sample

Table 2. Chrysotile asbestos recovery by TEM in the kidney cortex of control and test rats

Rat ID[b] and No.	Fiber count parameter[c]		Class of fibers A + B	A + B + C
	Total no. of fibers		B.D.[a] 10	
Control	Grid	Mean	B.D.	0.05
(4)	Fibers/hole (200)	Median	B.D.	0.04
	mg dry	Mean	B.D.	0.23×10^3
	Fibers/wt (200)	Median	B.D.	0.17×10^3
	Total no. of fibers		538	788
Test	Grid	Mean	0.87	1.15
(20)	Fibers/hole (881)	Median	0.42	0.74
	mg dry	Mean	3.80×10^2	5.34×10^3
	Fibers/wt (881)	Median	1.78×10^3	3.19×10^3

[a] B.D. = Below detection level
[b] Parenthetical numbers indicate number of animals under study
[c] Numbers in parentheses show number of grid holes examined

represented the highest dosage, since, the older the animal, the higher the cumulative dosage it would have. The statistical evaluation of transformed data by weighted regression analysis (Dixon 1975) revealed the following: When Class A + B fiber counts were considered, no linear or quadratic relationship was found at the 5% level of significance. However, when Class A + B + C fiber counts were considered, although there was no linear relationship between the age of animal and number of fibers, a quadratic relationship was found between the variables at $P < 0.08$ (Figure 5).

Fibers were at B.D. level in a lung specimen from one test specimen from one test rat whereas 4.1×10^2 fibers/mg dry wt. were recovered in the omentum. This concentration of fibers was 100% bundles. Bundles and clusters of fibers in kidney cortex were recovered in all age ranges of rats (Table 4) to form 25.8% of total fibers. Since the clusters contained one or more bundles, 57 bundles were encountered within 39 clusters. All bundles together formed 29.2% of total fibers. The number of fibrils

Table 3. Fiber counts in the kindey cortex from rats gavaged with chrysotile asbestos

Age group[a] of rats	Chrysotile fibers/mg dry wt. × 10^3 Class of fibers based on morphology[d]		
	A + B[b]	C	A + B + C
I	1.24 ± 1.1(B.D.)[c]	0.33 ± 0.3(B.D.)	1.57 ± 1.4(B.D.)
II	2.66 ± 1.7(B.D.)	2.71 ± 2.0(0.53)	5.37 ± 4.0(0.56)
III	8.00 ± 4.2(B.D.)	1.62 ± 0.6(B.D.)	9.63 ± 4.1(B.D.)
IV	3.31 ± 1.2(B.D.)	1.47 ± 0.9(0.34)	4.78 ± 2.0(0.34)

[a] Each group consisted of five rats
[b] Fiber counts in kidney cortex of control rats are shown in parentheses
[c] B.D. = Below detection level
[d] Fiber classes are defined in the text

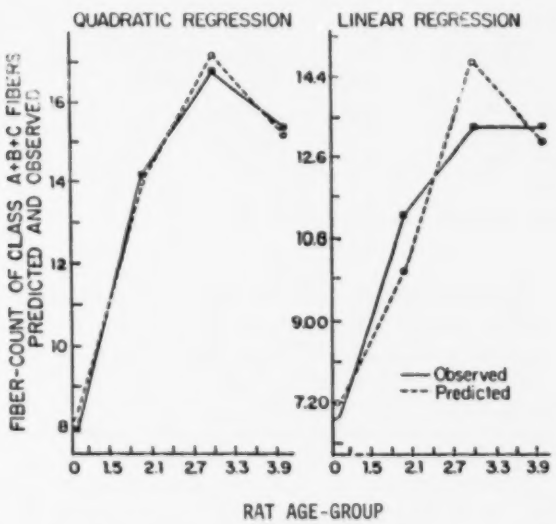

Fig. 5. Relationship between fiber count in kidney cortex and age (dosage) of rats in pooled test groups: Ordinate values represent SQRT transformation of Class A + B + C fiber counts.

shown in Table 4 includes the fibrils within clusters and fibrils by themselves.

Fiber dimensions were measured; however, only length data on A + B fibers are reported. Diameter was not meaningful, due to swelling or lack of sharp edges in some degraded fibers. Statistical information on length is shown in Table 5. The length varied from 0.08 to 5.71 μm, whereas the median ranged from 0.23 to 0.46 μm among the fibers recovered in four groups of rats. Frequency of length distribution (Table 6) of Class A and B fibers combined, was unimodal for rat groups I and III, was bimodal for rat group IV and trimodal for rat group II. The mean values of four groups showed bimodal distribution. Data derived from the fibers recovered in kidney cortex samples (N = 5) prepared from test rats in age groups II, III, and IV is shown in Table 7. There were a total of 54, 72, 162, and 34 fibers in Class A, B, C, and D, respectively. In each of the four classes, the highest number of fibers was in the length range 0.31–0.5 μm. Class C showed bimodal distribution with the modes located in length ranges 0–0.2 μm and 0.31–0.5 μm. The maximum fiber length in Class A, B, C, D was 2.64, 0.81, 0.61, 0.97 μm, respectively. Calculation of 50th percentile indicated that among Class A fibers 50% of fiber lengths fell short of 0.35 μm. This value was 0.34 μm for Class B fibers, and 0.24 μm for each of the Class C and Class D fibers. Frequencies of fiber lengths of the four classes of fibers recovered in samples from rat groups II, III and IV are presented in Figure 6.

Discussion

It is important to know whether ingested asbestos fibers penetrate the gastrointestinal tract, migrate, and are recovered in biological tissues and products. The literature is controversial. A number of studies have shown evidence of penetration (Westlake *et al.* 1965; Cunningham *et al.* 1976; Cunningham *et al.* 1977; Cook and Olson 1979; Hallenbeck and Patel-Mandlik 1978, 1979; Carter and Taylor 1980; Patel-Mandlik and Millette 1980; Sebastien *et al.* 1980). Negative conclusions have been reported by Gross *et al.* 1974; Bolton and Davis 1976; Hallenbeck *et al.* 1981. Human autopsy tissue analysis (Patel-Mandlik 1981) showed the presence of fibers in the kidney cortex. An additional interest in conducting this study was to answer the question: Is there a relationship between the amount of ingested chrysotile asbestos and concentration of fibers retained in the kidney cortex of chronically gavaged rats? Since the rats were gavaged twice every week the amount of ingested asbestos depended upon the age of rat. Thus, although the rate of gavage (50

Table 4. Fibrils, bundles, and clusters of chrysotile asbestos recovered in test samples[a]

Rat group	Fibrils	Bundles	Bundles within clusters	Clusters	Bundles as per cent of total fibers
I	38	11	4	3	28.85
II	75	15	13	7	27.18
III	155	46	15	10	28.24
IV	113	28	25	19	31.93
Total	381	157		39	29.18

[a] Values include only class A + B chrysotile

Table 5. Length distribution of chrysotile fibers[a] recovered in kidney cortex of gavaged rats

Rat group	No. of fibers	Length in micrometers						
		Max.	Min.	Mode	Median[b]	Mean	S.D.	Geo. mean
I	53	2.77	0.15	Not unique	0.35	0.46	0.47	0.96
II	103	5.40	0.11	0.38	0.46	0.99	1.21	1.15
III	216	2.23	0.11	0.38	0.35	0.43	0.32	0.96
IV	166	5.71	0.08	0.19	0.23	0.37	0.61	0.91

[a] Class A and B fibers only

[b] Median represents concept of an average better than the mean because of skewed distribution

mg/kg) was the same, the cumulative dose increased with increase in age.

Chrysotile fibers were recovered in the kidney cortex, which was selectively examined, showing that ingested fibers can penetrate the gastrointestinal tract and disseminate to a remote organ. Results are based on up to 250 observations per age group (age range) of rats since five rats were used per age range (0–200 days, 200–400 days, 400–600 days and 600–800 days) and 50 grid holes were examined per rat tissue. The results clearly indicated that fibers recovered in test samples were not from contamination, since animal exposure to airborne asbestos was minimal and contamination during sample preparation (blank grids) was B.D. level. One gavage study (Hallenbeck *et al.* 1981) failed to demonstrate penetration and migration of ingested fibers due to background (control) contamination counts in the same range as the counts recovered in tissues, blood, and urine of a test baboon. Moreover, the detection limit of their methodology was low and the results were based on analyses of tissues of a single test animal. Recovery of fibers in tissues of 17 out of 20 rats suggests that some animals may resist fiber penetration and migration. Biological variation was suggested by Sebastien *et al.* (1980) in their studies. Our study supports previous results (Hallenbeck and Patel-Mandlik 1979; Patel-Mandlik and Millette 1980) using different animal species, source of gavage material, and gavage conditions.

No fibers of Class A or B were encountered in the control tissues. Fibers of Class C were recovered in extremely low values (0.08 and 0.12 per grid hole) in two of the four control rat tissues. Statistical evaluation using ANOVA (Sokal and Rohlf 1973) on square root transformed data showed that the fiber concentrations in test and control tissues were significantly different ($P < 0.005$) when fibers of Class A + B were considered and were also significantly different ($P < 0.005$) with fibers of Class A + B + C. Comparisons with the control were made with each test group separately, as well as all combined test groups. The last section of this report discusses the findings on Class D fibers.

Comparison of the fiber concentrations within the four age groups (Table 3) showed a statistically significant difference ($P < 0.005$). A question about the dose-response relationship may be answered as follows: There was no linear relationship. Accepting statistical significance for P values less than 0.1, the quadratic model of regression analysis revealed a relation ($P < 0.08$) between dosage and fiber recovery based on Class A + B + C fiber counts. The extent of agreements of calculated fiber concentration with expected values is shown in Figure 5 as Observed and Predicted values. The values indicate a rise in fiber level followed by a drop, a lack of linear relationship. The quadratic relationship could signify time-related biological causes of low fiber recovery: degradation of fibers beyond TEM identity and elimination of fibers in feces and urine

Table 6. Frequency of length distribution of fibers recovered in kidney cortex of gavaged rats

Length interval (μm)	Per cent fibers[a] Rat group I	II	III	IV	Mean	Cumulative
0 −0.5	84.6	56.3	79.0	89.8	77.4	77.4
0.5−0.99	7.7	22.3	15.8	7.8	13.4	90.8
1.0−1.7	3.9	3.0	3.8	0.6	2.8	93.6
1.8−3.1	3.8	8.7	1.4	0	3.5	97.1
3.2−5.5	0	9.7	0	1.2	2.7	99.8
5.6−9.9	0	0	0	0.6	0.15	100.0

[a] Chrysotile fibers A + B sized by transmission electron microscopy

Table 7. Number of chrysotile fibers by Class A, B, C, and D in randomly selected grids

Grid no.	Rat group	Average no. of fibers/grid hole Class A	Class B	Class C	Class D
1	II	3.0	2.4	5.0	0.6
2	III	2.25	3.5	5.5	1.25
3	III	1.67	1.67	4.5	1.67
4	IV	0.26	2.55	5.55	1.27
5	IV	2.6	1.6	5.4	0.4
Mean	(N = 5)	2.03	2.34	5.19	1.04

(Cunningham *et al.* 1976; Hallenbeck and Patel-Mandlik 1979). No relationship was observed with A + B fiber counts.

Length of chrysotile asbestos Class A + B fibers recovered in test tissues showed skewed distribution. Shorter fibers outnumbered the longer fibers. All the fibers were less than 9 μm in length (Table 5). It is not known whether shorter fibers selectively penetrate and disseminate or the fibers break into shorter lengths during the journey from oral entry to kidney cortex through the blood system. Plastibest-20 chrysotile fibers of which gavage material NIEHS 109c was a sample, had approximately 65% of fibers that exceeded 10 μm in length (Campbell *et al.* 1980) as measured by scanning electron microscopy. Table 5 showing length distribution, by transmission electron microscopy, of NIEHS 109c chrysotile fibers retained in kidney cortex indicated all fibers were shorter than 10 μm. The health significance of a large volume of short length-retained fibers is not known. Hamilton *et al.* (1981) reported that a sample containing chrysotile fibers shorter than 0.5 μm was ineffective in coagulation of plasma. There was no particular pattern of fiber length in relation to the age group of rats (Table 5) except that fibers recovered in Group II tissues were lower in number in the length range 0−0.5 μm compared to the other groups and showed trimodal distribution. Morphological changes demonstrated in Figures 1−4 may be representative of a sequence of alterations occurring in chrysotile morphology during its passage in the biological system. Fiber classes A, B, C, and D were formed arbitrarily depending upon the extent of degradation. In general, chrysotile fibers in a degraded state did not show characteristic tubular appearance and/or a visible central canal but provided enough identity to permit one to say that they were chrysotile asbestos. It was not possible within the scope of this study to use microprobe analysis for more precision. Degraded fibers have been previously reported (Hallenbeck and Patel-Mandlik 1979; Patel-Mandlik and Millette 1980) in gavage studies. It is suggested that there might be a degradative mechanism associated with the residence time of fibers *in vivo*. It is possible that during their passage and stay within the body system, fibers could be influenced by the body fluids, temperature, agitation, and other factors.

Information on the proportion of each class of fibers recovered in relation to the age group of rats (dosage) and to the frequency of fiber length distribution was interesting. The results shown in Table 7 and Figure 6 were generated from data on 322 fibers recovered in the grids examined by the U.S. EPA laboratory at Cincinnati, Ohio. The mean number of fibers per grid hole was the highest for Class C and the lowest for class D fibers. The values for Class A and Class B fibers were approximately similar (Table 7).

Since the majority of fibers recovered in the kidney cortex were less than 0.5 μm long (Table 5), three length ranges were formed within the size 0 to 0.5 μm (Figure 6). The maximum number of fibers recovered (in each of the four fiber classes) were in the length range of 0.31 to 0.5 μm. The frequency of length distribution of Class A, B, C, and D fibers recovered in each of the age groups II, III, and IV is illustrated in the nomograph. Group I was excluded, since the fiber recovery in these tissues was low as shown before (Table 3).

Assuming that degradative mechanisms affecting the morphology and diffraction property of the chrysotile preclude the positive identification of all of the fibers present on the grid surface, the results expressed in fibers/mg. dry wt. could be considered to be conservative measures of tissue fiber concentration. Mass concentration was not computed, since it is often very inaccurate because of poor counting statistics associated with large fibers (bun-

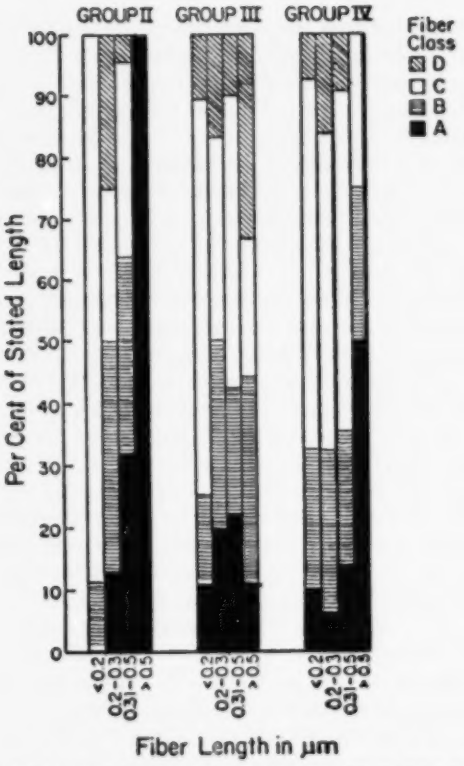

Fig. 6. Frequency of length distribution of chrysotile fibers among their four morphological classes: A, B, C, and D

dles, clusters) that are fewer in number but represent most of the actual mass. Recovery of bundles and clusters suggests that besides single fibrils other forms could penetrate and migrate or that fibers accumulate in the same area of the tissue or that the preparation procedure causes some fibers to clump together.

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